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Meta-analysis of genome wide association studies (GWAS) on the intolerance of Angiotensin converting enzyme inhibitors

Running head: Meta-analysis of GWAS on intolerance of ACE-inhibitors

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Abstract:

Objectives: To identify SNPs associated with switching from an ACE-inhibitor to an angiotensin receptor blocker (ARB).

Methods: Two cohorts of patients starting ACE-inhibitors were identified within the Rotterdam Study in the Netherlands and the GoDARTS study in Scotland. Cases were intolerant subjects who switched from an ACE-inhibitor to an ARB, controls were subjects who used ACE-inhibitors continuously for at least 2 years and did not switch. GWAS using an additive model was run in these sets and results were meta-analysed using GWAMA.

Results: 972 cases out of 5 161 ACE-inhibitor starters were identified. 8 SNPs within 4 genes reached the GWAS significance level ($P<5\times10^{-8}$) in the meta-analysis (*RBFOX3*, *GABRG2*, *SH2B1* and *MBOAT1*). The strongest associated SNP was located in an intron of *RBFOX3*, which contains a RNA binding protein (rs2061538: MAF=0.16, OR=1.52[95%CI: 1.32-1.76], $p=6.2\times10^{-9}$).

Conclusions: These results indicate that genetic variation in abovementioned genes may increase the risk of ACE-inhibitors induced adverse reactions.

Keywords: ACE inhibitors, ACE-inhibitor intolerance, adverse drug reaction, cough, angioedema, Genome Wide Association Study.

Introduction:

Angiotensin converting enzyme inhibitors (ACE-inhibitors) are one of the most frequently prescribed groups of medications for the management of high blood pressure, heart failure and renal disease [1]. While ACE-inhibitors are generally prescribed for lifetime treatment, a cohort study showed that 32.4% of patients halted their medication likely due to adverse drug reactions (ADRs) within a median 336 days follow up time [2]. The most common ACE-inhibitor induced ADR is a persistent, dry cough and the most severe one is life threatening angioedema of lips, tongue and upper airway [3]. There is evidence suggesting genetic predisposition to these ADRs; ACE-inhibitor induced cough occurs with higher incidence in East Asian patients (23%) compared with Caucasians (5–11%) [4, 5]. The ACE-inhibitor induced angioedema rate is higher in black patients than in white patients and angioedema patients often have affected relatives [6, 7].

The mechanism of ACE-inhibitor induced cough and angioedema is not completely understood. ACE-inhibitors inhibit Angiotensin I Converting Enzyme (ACE) that cleaves several target proteins including angiotensin I and pro-inflammatory kinins. The blood pressure modification takes place through angiotensin I [8]. Accumulation of these inflammatory kinins is hypothesized to be the main reason of ACE-inhibitor induced angioedema and cough [9, 10]. For two decades, multiple candidate genes studies have tested the associations between ACE-inhibitor induced cough and genetic variation in ACE and bradykinin pathways, of which the insertion-deletion (I/D) variation in the ACE gene has been investigated most frequently [11-14]. A meta-analysis of 12 such studies, did not find a statistically significant association for the

ACE I/D polymorphism [15]. Studies on ACE-inhibitor induced angioedema have also been conducted with the same approach; 3 of them found a statistically significant association between ACE-inhibitor induced angioedema and single nucleotide polymorphisms (SNPs) in the *XPNPEP2* gene [16-18]. One study showed that the bradykinin receptor2 (B2) -9/+9 polymorphism is associated with both ACE-inhibitor induced cough and angioedema [19]. However generally, most of the candidate gene approach studies have been difficult to replicate and their results should be interpreted with caution [20]. The only genome wide association study (GWAS) on 175 ACE-inhibitor induced angioedema cases and 489 controls that also used ACE-inhibitors, found no genome-wide association, which might be due to the small sample size [21]. For ACE-inhibitor induced cough, the only GWAS with 1 595 cases and 5 485 controls identified genome-wide significant associations in *KCNIP4* gene at chromosome 4 (rs145489027, p=1.0x10-8) which was replicated in 2 independent populations [22].

Based on the probable similar mechanism of ACE-inhibitor induced ADRs (cough and angioedema), this study aims to use a GWAS approach to identify SNPs associated with intolerance of ACE-inhibitors defined as switching of an ACE-inhibitor to an angiotensin receptor blocker (ARB) as a marker for ADRs [23].

Methods:

Study population:

This study was performed in 2 separate European populations:

A) The Rotterdam study in the Netherlands has been described in detail previously [24, 25]. In summary, it is an ongoing cohort, composed of three different sub-cohorts (RS1, RS2, and RS3), started in 1990 in Ommoord a suburb of Rotterdam that has included 14 926 subjects aged 45 years or older (72.0 % of 20 744 eligible invited people). The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek: ERGO (Population Study Act: Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants gave informed consent to participate in the study and to obtain information from treating physicians and pharmacies, separately.

B) The (Go-DARTS study) which is a genetic sub-study of The Diabetes Audit and Research Tayside, Scotland (DARTS) that has been described and validated in previous publications [26]. In summary, this project was based on linking clinical records by a patient-specific identifier, allowing the creation and maintenance of sophisticated regional health informatics systems. The DARTS project electronically followed all residents in Tayside, since January 1996 (n=391 274 including 7 596 individuals with diabetes) through linking the clinical datasets with a high degree of reliability and accuracy. Collection and analysis of data in DARTS and Go-DARTS was approved by the East of Scotland Research and Ethics Committee, in compliance with the declaration of Helsinki.

Phenotype:

For both study populations similar phenotype definitions were applied for cases and control selection:

Cases: Patients who switched to an ARB during ACEI treatment.

Controls: Patients, who started ACE-inhibitors, and continued treatment for at least 2 years. They did not discontinue or switch their ACE-inhibitors during the follow up.

For defining continuation, discontinuation or switching, a maximum of 6 months gap between 2 prescription periods was considered. These definitions were validated in our previous study as the best marker of ACE-inhibitor induced ADRs within the prescription databases [23].

Genotyping:

Within the Rotterdam study a total of 12 453 subjects were genotyped with Illumina 500(+duo) and Illumina 610 quad and 11 496 subjects passed genotyping quality control. Exclusion criteria for SNPs were a call rate <98%, Hardy-Weinberg p-value <1 × 10^-6, minor allele frequency <0.01%, excess autosomal heterozygosity >0.336, sex mismatch and outlying identity-by-state clustering estimates. Data was imputed with the 1000-Genomes reference panel (phase 1, version 3) using MACH version 1.0.15/1.0.16.

Within the Go-DARTS study, subjects were genotyped on the Affymetrix 6.0 (Affymetrix, Santa Clara, CA, USA) or Illumina HumanOmniExpress (Illumina, San Diego, CA, USA) platforms. Both platforms were imputed using IMPUTE2 and the 1000 Genomes reference panel. SNPs deviating from Hardy–Weinberg equation (P<1 × 10^−6) or with an Info Score <0.4 were excluded.

Data analyses:

The primary single SNP tests of association were performed using logistic regression assuming an additive genetic model, adjusting for age and gender. PLINK v1.07 was used for the Dutch cohort [27] and SNPTEST-v2.5-beta was used for the Scottish cohort [28]. The fixed effect meta-analyses were done at both sites using the inverse variance weighting, in the Netherlands using METAL and Scotland using GWAMA [29, 30]. The final SNP list in the Netherlands analysis was filtered based on the index of heterogeneity (I^2 <60) and the number of cohorts that covered a SNP (more than two cohorts) [31]. The final values presented in this study are from the analyses in Scotland because GWAMA provides the odds ratios and does not require further calculations; however the consistency of the results at both sites was considered for the most significantly associated SNPs. Data of SNPs around the most significant gene were visualized using LocusZoom [32]. All other analyses were performed using SAS v9.3 (SAS Institute, Cary, NC, USA). R packages were used to plot the graphs. Metafor R package used for forest plot [33] and qqman package for Manhattan and QQ plot [34].

Results:

A total of 710 cases of ACE-inhibitor intolerant patients and 3 599 tolerant controls in the Genetics of Diabetes Audit and Research in Tayside Scotland (Go-DARTS) population and 262 cases and 590 controls in the population of the Rotterdam study were analysed separately and subsequently meta-analysed. 2004 patients from Go-DARTS population were genotyped using the Illumina chip (GD1) and the rest (2305 patients) were genotyped using the Affymetrix chip (GD2). Three sub populations within Rotterdam study, RS1, RS2 and RS3 had 630, 170 and 52 patients respectively). In both cohorts the mean age of included patients was not statistically

significantly different between cases and controls. The proportion of females was significantly higher within cases compared to controls in both cohorts (Table 1).

In the meta-analysis of both cohorts using multivariable regression analyses adjusting for gender and age, 8 SNPs located on chromosome 5 (one SNP), 6 (one SNP), 16 (one SNP) and 17 (five SNPs) reached genome-wide significance level (P-value less than $5x10^{-08}$) (Figure 1 and 2). Table 2 shows the details of the most statistically significantly associated SNPs. From these SNPs, two were only available in the Go-DARTS population (rs192613545 and the insertion/deletion polymorphism on chromosome 17 position 77112502). A List of the most significantly associated SNPs which reached P-value of less than 10^{-05} in meta-analysis, is available in the supplement in Table 1. The most significantly associated SNP (rs2061538) was located within the gene *RBFOX3* (RNA Binding Protein, Fox-1 Homolog (C. Elegans) 3). There were several other strongly associated SNPs in high linkage disequilibrium (LD) with this SNP in that region (Figure 3A). The second most statistically significant SNP (rs77370934) was located within the gene *GABRG2* (Gamma-Aminobutyric Acid Receptor Subunit Gamma-2), however, there were no other SNPs with a high level of LD in that locus (Figure 3B).

There were also genome wide statistically significant SNPs within the *MBOAT1* gene (Membrane Bound O-Acyltransferase Domain Containing 1) and *SH2B1* gene (SH2B Adaptor Protein 1).

Figure 4 presents the odds ratio and the 95% confidence interval (95% CI) for the two most statistically significantly associated SNPs for the different sub studies of the Rotterdam study

and the Go-DARTS population. Except for the RS3 which is the smallest subpopulation, the effect directions were concordant between the populations.

A high level of consistency was observed for the meta-analyses results from both sites using the GWAMA and METAL, particularly for the most significantly associated SNPs.

Discussion:

Our study describes a large GWAS study investigating SNP variants associated with switching of an ACE-inhibitor to an ARB as a marker for ACE inhibitor induced ADRs. All phenotype data for this study were derived from clinical settings that incorporate either the prescription data system (GoDARTS) or the pharmacy drug dispensing database (Rotterdam study). We found statistically significant associations with SNPs located within the genes *RBFOX3, GABRG2, SH2B1 and MBOAT1*. These are novel candidate genes which may play a role in the adverse drug reactions to ACE-inhibitors.

The SNPs showing the strongest association with the phenotype are located on chromosome 17 within the gene *RBFOX3*. This is a member of the *RBFOX* family that in mammals consists of three members: *RBFOX1, RBFOX2* and *RBFOX3. RBFOX3* is expressed specifically in neuronal cells. This protein contains an RNA recognition motif that binds specifically to an RNA element, UGCAUG and regulates alternative pre-mRNA splicing. Alternative splicing of pre-mRNA is an important mechanism for post-transcriptional regulation of gene expression and has increasingly been appreciated as a major mechanism to generate diversity of gene products in higher eukaryotes [35, 36].

The other most strongly associated SNP was located on chromosome 5 within the gene *GABRG2* which encodes a gamma-aminobutyric acid (GABA) receptor. GABA is the major inhibitory neurotransmitter in the mammalian nervous system, where it acts at GABA-A receptors. GABA-A receptors are pentameric, consisting of proteins from several subunit classes: alpha, beta, gamma, delta and rho [37]. There are several studies proving the effects of GABA receptor agonists in decreasing the sensitivity to cough both in animal models and in humans. This makes them a possible target for cough treatment [38]. Dicpinigaitis *et al* showed that Baclofen (as a GABA receptor agonist) can supress cough induced by ACE-inhibitors [39]. They also proved in a prospective clinical trial that baclofen can inhibit capsaicin-induced cough [40].

SH2B1 (sarcoma (Src) homology 2 (SH2) B adaptor protein 1) is a member of a family of scaffold proteins implicated in signalling downstream of a variety of receptor tyrosine kinases and cytokine receptors [41]. Variations in this gene have been reported to be associated with obesity [42]; however its role in the abnormal glucose homeostasis has not been proved [43]. The significant association of this gene with the intolerance of ACE-inhibitors needs to be further investigated because there was no previous report of this gene contributing in cough or angioedema.

MBOAT1 (membrane bound O-acyltransferase domain containing 1) belongs to the superfamily of *MBOAT* that transfer organic compounds, usually fatty acids onto hydroxyl groups of membrane-embedded targets [44]. This trans-membrane protein has been reported to be involved in developmental processes [45].

The main hypothesized mechanism of ACE-inhibitor induced ADRs (mainly cough and angioedema) is stimulation of sensory nerve resulting from the accumulation of inflammatory mediators that are normally cleaved by the ACE [3]. This hypothesis has served as the basis for candidate gene studies that have focused on variation in inflammatory pathways, however findings of those candidate gene studies were replicated inconsistently and the meta-analyses of loci that had enough studies, did not find the significant effect for the insertion/deletion polymorphism within ACE gene [15]. Hypothesis free GWA studies may lead to finding novel loci to be associated with ADRs of ACE-inhibitors. The only available large GWAS on ACE-inhibitor induced cough found an association with Kv Channel Interacting Protein 4 (*KCNIP4*) which is predominantly expressed in nervous systems [22]. However the only available GWAS on the ACE-inhibitor induced angioedema with 175 ACE-inhibitor induced angioedema cases and 489 controls could not find any significant association on a genome wide level which could be due to the relatively small sample size and lack of the power [21]. Our results suggest that an important source of variation may be directly related to the sensory nerves themselves, because both *GABRG2* and *RBFOX3* genes are playing a role in the central and peripheral nervous systems as well. These findings are in line with the previous GWAS on ACE-inhibitor induced cough [22].

This study is a large GWAS on the intolerance of ACE-inhibitors within a population of European ancestry. However the direct relevance of our findings with ACE-inhibitor induced ADRs is not clear yet and needs to be further investigated, these findings, if replicated in other populations, can improve our understanding of the biological mechanism of ACE-inhibitor induced ADRs. Furthermore, it will help to identify those patients at high-risk to develop ACE-inhibitor induced

ADRs including angioedema, which is a life threatening event. We recently showed that approximately 50% of ACE-inhibitor users continue ACE-inhibitors after the first episode of angioedema [46]; Identification of those patients at high risk could help physicians guide their treatment choice. ACE-inhibitor induced cough is not as life threatening as angioedema but it can be misdiagnosed and mistreated which significantly decreases the compliance of patients and might finally result in unsuccessful drug therapy [47, 48]. Therefore in the context of precision medicine, the ultimate application of these findings within the clinic would be the prediction of susceptible patients and treating them with an alternative medication with comparable effect such as ARBs [49].

An important limitation of this study is defining phenotype based on the electronic medical records which can potentially lead to misclassification of cases and controls. However in a validation study, the proxy marker for cases showed a positive predictive value of 68.3% for probable ACE-inhibitor induced ADRs [23]. This study also cannot detect associations for rare SNPs (MAF< 0.01%). The study results are restricted to the European ancestor populations.

In conclusion, this study used a GWAS to identify SNP variants associated with ACE-inhibitor intolerance as a marker of ADRs. We identified SNPs in the genes RBFOX3, GABRG2, SH2B1 and MBOAT1 as potential candidates for ACE inhibitor induced ADRs. Due to the fact that this is a hypothesis generating study, the functional role of significantly associated genes was not investigated; therefore future studies are needed to replicate our findings in addition to the epigenetic and molecular studies are needed to explore the functional roles of variations within genes reported in this study specifically the *GABRG2* gene for which several clinical studies also

showed its role in susceptibility to cough [38-40]. The standard clinical criteria have been described for ACE-inhibitor induced angioedema [50] and to make it possible to combine results it would be good if new genetic association studies would use this standard phenotype in the future.

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FIGURE LEGENDS

Figure 1. Manhattan plot of genotyped SNPs associated with ace-inhibitor intolerance using an additive model adjusted for age and gender. The red line indicates the genome-wide significance threshold of alpha=$5x10^{-8}$.

Figure 2. A QQ plot for SNP associations from a meta-analysis of GWAS of ACE-inhibitor intolerance using an additive model adjusted for age and gender. (Lambda=0.88)

Figure 3. LocusZoom plot of most strongly associated SNPs from the meta-analysis located in A) the region of most significantly associated genes

A) The *RBFOX3* (chromosome 17 centred around SNP rs2061538 (shown in purple). Linkage disequilibrium (based on r^2 values) with respect to rs2061538 are based on the CEU reference population.
B) The *GABRG2* (chromosome 5 centred around SNP rs77370934 (shown in purple). Linkage disequilibrium (based on r^2 values) with respect to rs77370934 are based on the CEU reference population.

Figure 4. The forest plot from the meta-analyses of most strongly associated SNPs

GD: GoDARTS, RS: Rotterdam study, CI: confidence interval